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Structure–function relationships of human JmjC oxygenases — demethylases versus hydroxylases

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The Jumonji-C (JmjC) subfamily of 2-oxoglutarate (2OG)-dependent oxygenases are of biomedical interest because of their roles in the regulation of gene expression and protein biosynthesis. Human JmjC 2OG oxygenases catalyze oxidative modifications to give either chemically stable alcohol products, or in the case of N^{ε}-methyl lysine demethylation, relatively unstable hemiaminals that fragment to give formaldehyde and the demethylated product. Recent work has yielded conflicting reports as to whether some JmjC oxygenases catalyze N-methyl group demethylation or hydroxylation reactions. We review JmjC oxygenase-catalyzed reactions within the context of structural knowledge, highlighting key differences between hydroxylases and demethylases, which have the potential to inform on the *possible* type(s) of reactions catalyzed by partially characterized or un-characterized JmjC oxygenases in humans and other organisms.

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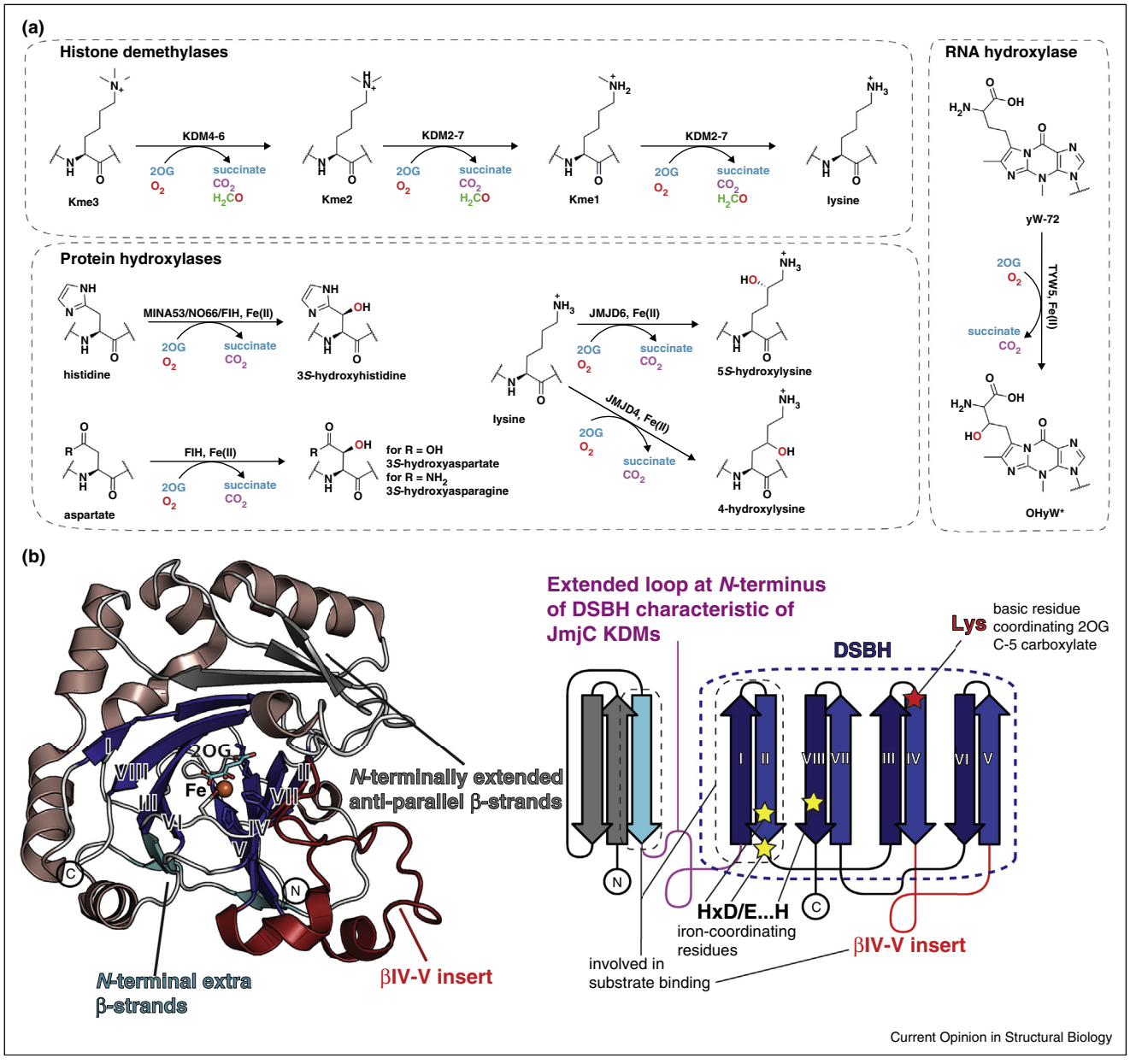
Introduction

Interest in human Fe(II)- and 2-oxoglutarate (2OG)-dependent oxygenases has been stimulated by the discovery of their roles in regulation of gene expression and protein biosynthesis. More than 60 human 2OG oxygenases have been identified, with roles in diverse processes such as fatty acid metabolism (including carnitine biosynthesis), collagen biosynthesis, development, and the hypoxic response [1]. Of these, the Jumonji-C domain-containing oxygenases (JmjC oxygenases) have attracted attention, including from a pharmaceutical perspective [2], principally because of their roles in histone demethylation [3•,4•,5•,6], though they have wider roles in protein hydroxylation [7•,8•,9]. The JmjC oxygenases are a subfamily of 2OG oxygenases that share sequence homology and structural similarities (as detailed below). Most JmjC oxygenases (>20) have been assigned as histone lysine demethylases (KDMs), which catalyze N^{ε}-methyl lysine residue demethylation reactions (Figure 1a), proceeding via initial N^{ε}-methyl group hydroxylation followed by fragmentation to give the demethylated product and formaldehyde. Other members of the JmjC subfamily catalyze oxidation of proteins (and, in one case, tRNA) to give a stable hydroxylated product; we refer to these as 'JmjC hydroxylases'. This dual functionality within the JmjC subfamily has led to conflicting reports regarding the assignment of some JmjC oxygenases. To date, several JmjC oxygenases have been reported to catalyze both demethylation and hydroxylation. Here, we review JmjC oxygenases from structural and biochemical perspectives, focusing on members that have been structurally characterized. We discuss structural features common to different types of JmjC oxygenases (i.e. hydroxylases versus demethylases) and correlate these with the types of reactions that they catalyze.

History and overview of the JmjC oxygenases

The JmjC domain is named after the 'Jumonji' protein in which it was first identified [10]. The name 'Jumonji' (meaning 'cruciform' in Japanese) is based on the abnormal 'cross-like' appearance of neural grooves in *Jumonji* (*Jmj*)-null mice. The *Jmj* gene was proposed to encode for a putative Jumonji protein (corresponding to human JARID2) [10], which contains a DNA-binding domain (AT-rich interaction domain, ARID), JmjC, and Jumonji-N (JmjN) domains [11]. The latter two were proposed to co-occur [11], though later work revealed they are separate structural and functional units [12]. Bioinformatics indicated that the JmjC domain is widely distributed in eukaryotes [11,12]. Since many of the proteins containing JmjN and JmjC domains had been previously linked with transcriptional control and contained known DNA binding domains (e.g. plant homeobox domain (PHD), ARID, or Zn fingers), they were collectively termed the Jumonji family of transcription factors [11]. Bioinformatics identified that the JmjC domain shared considerable similarity

Figure 1



Characteristic features of JmjC domain architecture and reaction catalysis. **(a)** Demethylation and hydroxylation reactions catalyzed by human JmjC oxygenases. **(b)** (*Left*) View from a crystal structure of an FIH monomer (PBD ID: 1H2K, [14•]) as a representative JmjC oxygenase, and (*right*) two dimensional domain topology of a generalized JmjC domain (generated using TopDraw). The JmjC domain fold contains a distorted double-stranded β-helix (DSBH; β-strands I–VIII; *blue*) fold, additional *N*-terminal anti-parallel β-strands that extend the DSBH (*grey*), and a βIV-V insert (*red*). Fe(II) (*orange sphere*) and 2OG (*cyan sticks*) are bound in the active site. Abbreviations are defined in Table 1.

with enzymes containing a cupin or double-stranded β-helix (DSBH) fold, leading to the proposal that JmjC proteins act as Zn(II)-dependent transcriptional regulators, since phosphomannose isomerase is a Zn(II)-utilizing DSBH fold enzyme [12].

Studies on the first biochemically and structurally characterized JmjC oxygenase, factor inhibiting hypoxia inducible factor (FIH), reveal the JmjC domain indeed adopts a DSBH fold [7•,8•,13•,14•,15•]. Rather than supporting a Zn(II)-binding active site, FIH is an Fe(II)- and 2OG-dependent oxygenase like many JmjC enzymes. This work identified roles for JmjC oxygenases in transcriptional regulation via FIH-catalyzed asparaginyl hydroxylation of hypoxia inducible factor-α (HIF-α) isoforms [7•,8•], a reaction important in the hypoxic response; FIH regulates HIF

activity by reducing its binding to transcriptional coactivators (CBP/p300) [16]. These discoveries were followed by important work identifying the first JmjC domain-containing KDMs, that is the KDM2, 3, and 4 subfamilies [3•,4•,5•]. These studies expanded roles of JmjC oxygenase catalysis to 'epigenetic' regulation and identified JmjC KDMs as the largest class of histone KDMs [6], with the flavin-dependent lysine specific KDMs constituting a smaller class [17]. Structural studies of the JmjC catalytic core of KDM4A provided the first insights into KDM structure–function relationships [18•,19•,20•]. Since the identification of FIH and the first JmjC KDMs, the substrate repertoire of JmjC oxygenases has expanded to include other KDMs acting on histone tails [6], multiple non-HIF-α FIH substrates (mostly ankyrin repeat domain proteins) [21], mRNA splicing factors [22•], tRNA [23•], ribosomal proteins [24•], and other proteins involved in translation [25•] (Table 1). These discoveries reveal 2OG oxygenase-catalyzed hydroxylation of proteins is more extensive than was perceived before the identification of JmjC oxygenases [9].

There are controversies regarding functional assignments of some JmjC oxygenases: Jumonji domain-containing 6 (JMJD6) is proposed to catalyze both N-methyl argininyl demethylation and lysyl hydroxylation [22•,26•,27–29], the ribosomal oxygenases (ROXs), MYC-induced nuclear antigen 53 (MINA 53) and nucleolar protein 66 (NO66) are assigned as both KDMs [30,31] and ribosomal protein hydroxylases [24•], and there are conflicting proposals for Jumonji domain-containing 5 (JMJD5) as either a KDM or hydroxylase [32,33].

JmjC domain architecture and catalysis

All structurally characterized 2OG oxygenases, including JmjC oxygenases, possess a distorted DSBH core fold comprising eight anti-parallel β-strands (labeled I–VIII in Figure 1b; Supplementary Figure 1) [34]. The DSBH fold characterizes both cupin and JmjC proteins; the different nomenclature has emerged likely for historical reasons. Note there is no absolute definition of a JmjC protein; however, detailed bioinformatics has previously highlighted sequence and structural features that distinguish the JmjC oxygenases from the larger 2OG oxygenase superfamily [35•].

The asymmetric, barrel-like DSBH fold of 2OG oxygenases is formed from long β-strands I, VIII, III and VI (major β-sheet) on one face and short β-strands II, VII, IV and V (minor β-sheet) on the other (Figure 1b). The DSBH supports several conserved active site residues including those binding Fe(II) and 2OG. Fe(II) is normally, but not always, bound by an HxD/E···H triad, in which Asp is more common than Glu; the only human JmjC oxygenases with a Glu residue are KDM4 and KDM6 subfamily members and JMJD1C [34,36]. The first His of the triad is situated at the end of βII, with the Asp/Glu normally located on the βII/βIII loop, while the 'distal' His is positioned at the start of βVII, spatially adjacent to βII (Figure 1b). 2OG binds to Fe(II) in a bidentate manner via its C-2 keto and C-1 carboxylate groups (Supplementary Figure 2A); its binding is stabilized by electrostatic and hydrogen bonding interactions, including between its C-5 carboxylate and a basic residue located deeper in the DSBH. For human JmjC oxygenases, this residue is typically a lysine located at the βIV *N*-terminus. Exceptions to this include the KDM6 subfamily [37–39], where the lysine is on βI, and KDM3B, where the lysine is on βVIII (PBD ID: 4LXL). The bacterial JmjC hydroxylase ycfD is the only structurally characterized JmjC oxygenase with an arginine rather than a lysine residue on the βIV strand [40,41•], a difference proposed to reflect changes in JmjC evolution [41•]. As for other 2OG oxygenases, the JmjC oxygenases appear to use an ordered sequential mechanism, where 2OG binding is followed by substrate and, finally, O_2 (Supplementary Figure 2B) [42]. Oxygen binding enables 2OG decarboxylation (giving succinate and CO_2), yielding a reactive Fe(IV)=O species that effects substrate oxidation [42]. In the case of N-methyl demethylation, hydroxylation results in a hemiaminal, which fragments to form the demethylated product and formaldehyde [3•,5•,43]. Release of the hydroxylated/demethylated product and succinate completes the cycle [42]. While the overall 2OG oxygenase superfamily catalyzes a very diverse set of reactions [1], JmjC catalysis is currently limited to hydroxylation and demethylation (Figure 1a) [9].

Overall domain architecture and roles of non-catalytic domains

Though the JmjC oxygenases have conserved catalytic domains, their overall architectures vary. Most JmjC KDMs have multiple domains and are typically 2–3 times longer than the JmjC hydroxylases (Table 1). The additional non-catalytic domains in KDMs are subfamily-characteristic histone-binding and nucleic acid-binding domains, some of which enable targeting to specific (sets of) histone modifications. For example, KDM7B-catalyzed demethylation of di/monomethylated histone H3 lysine-9 (H3K9me2/1) is strongly promoted by pi-cation-pi/hydrophobic interactions between an aromatic cage in its PHD domain and H3K4me3 [44•] (Figure 2). In the case of KDM4A, which catalyzes demethylation of H3K9me3/2, H3K36me3/2, and H1.4K26me3/2, its tudor domains bind histones via interactions between an aromatic cage and H3K4me3 and H4K20me3 sites [45–47]. The JmjC hydroxylases appear to lack chromatin-associated domains, but they have other domains/regions, such as dimerization and winged-helix (WH) domains (Table 1), that can be involved in substrate binding. A hydrophobic region in the FIH dimerization domain promotes HIF-α substrate binding (Figure 2) [14•]. Similarly, the dimerization domain of the JmjC nucleic acid hydroxylase, tRNA wybutosine-synthesizing protein 5 (TYW5), is proposed to play a role in tRNA binding, in

Table 1

Reported biochemical functions of human JmjC oxygenases.

	Subfamily	Name	Other Names	NCBI/ UniProt ID	Catalytic Function	Reported Biochemical & Cellular Substrate(s)	Domain Architecture†
Histone Demethylases	KDM2 (FBXL)	KDM2A*	FBXL11; JHDM1A	22992/Q9Y2K7	Lys(me2/1) demethylase	H3K36, p65	1162
		KDM2B	FBXL10; JHDM1B	84678/Q8NHM5	Lys(me2/1) demethylase	H3K36, H3K4me3	
	KDM3 (JMJD1)	KDM3A*	JMJD1A; JHDM2A	55818/Q9Y4C1	Lys(me2/1) demethylase	H3K9	1321
		KDM3B	JMJD1B; JHDM2B	51780/Q7LBC6	Lys(me2/1) demethylase	H3K9	
		JMJD1C	-	221037/Q15652	Lys(me2/1) demethylase	H3K9, MDC1	
	KDM4 (JMJD2)	KDM4A*	JMJD2A; JHDM3A	9682/O75164	Lys(me3/2/1) demethylase	H3K9, H3K36, H1.4K26	1064
		KDM4B	JMJD2B; JHDM3B	23030/O94953	Lys(me3/2/1) demethylase	H3K9, H3K36, H1.4K26	
		KDM4C	JMJD2C; JHDM3C	23081/Q9H3R0	Lys(me3/2/1) demethylase	H3K9, H3K36, H1.4K26; Pc2	
		KDM4D*	JMJD2D; JHDM3D	55693/Q6B0I6	Lys(me3/2/1) demethylase	H3K9, H3K36, H1.4K26	523
		KDM4E	JMJD2E	390245/B2RXH2	Lys(me3/2/1) demethylase	H3K9	
	KDM5 (JARID)	KDM5A* KDM5B KDM5C KDM5D	JARID1A; RBP2 JARID1B; PLU1 JARID1C; SMCX JARID1D; SMCY	5927 P29375 10765/Q9UGL1 8242/P41229 8284/Q9BY66	Lys(me3/2/1) demethylase	H3K4	1690
	KDM6	KDM6A*	UTX	7403/O15550	Lys(me3/2/1) demethylase	H3K27	1401
		KDM6B‡	JMJD3	23135/O15054	Lys(me3/2/1) demethylase	H3K27	
		KDM6C	UTY	7404/O14607	Lys(me3/2/1) demethylase	H3K27	
	KDM7	KDM7A	KIAA1718; JHDM1D	80853/Q6ZMT4	Lys(me2/1) demethylase	H3K9, H3K27, H4K20	1060
		KDM7B*	PHF8; JHDM1F	23133/Q9UPP1	Lys(me2/1) demethylase	H3K9, H4K20	
		KDM7C	PHF2; JHDM1E	5253/O75151	Lys(me2/1) demethylase	H3K9, H3K27, H4K20me3, ARID5B	
Controversial Assignments	JmjC-only	JMJD6	-	23210/Q6NYC1	Lys (*5S*)-hydroxylase; Arg(me1) demethylase**	Splicing proteins (e.g. U2AF65, LUC7L2), p53 H3R2**; H4R3**	403
		MINA53	MINA	84864/Q8IUF8	His (*3S*)-hydroxylase; Lys(me3/2) demethylase**	RPL27; H3K9**	
		NO66	-	79697/Q9H6W3	His *3S*-hydroxylase; Lys(me3/2) demethylase**	RPL8; H3K4**, H3K36**	465
		JMJD5	KDM8	79831/Q8N371	Lys(me2) demethylase** Hydroxylase**	H3K36** NFATc1**	416
Protein/nucleic acid hydroxylases	JmjC-only	FIH	HIF1AN	55662/Q9NWT6	Asn (*3S*)-hydroxylase; Asp/His (*3S*)-hydroxylase	HIF-1α; Multiple ankryin repeat domain proteins	349
		JMJD4	-	65094/Q9H9V9	Lys C-4 hydroxylase	eRF1	463
		TYW5	-	129450/ A2RUC4	Wybutosine β-hydroxylase	$tRNA^{Phe}$ (yW-72)	315
Uncharacterized JmjC oxygenases	JmjC-only	HSPBAP1	PASS1	6767/Q96EW2	n/d	n/d	488
		JMJD7	-	100137047/ P0C870	n/d	n/d	316
		JMJD8	-	339123/Q96S16	n/d	n/d	334

JmjC JmjN F-box LRR CXXC Zn Finger C6 Zn Finger PHD Zn Finger ARID TPR Tudor Dimerization WH

* Subfamily domain architecture representation is based on this enzyme.

** Controversial functional assignments.

† Domain architecture based on reported domains as defined by PROSITE-ProRule [74] or structures (MINA53 [41•], FIH [14•], and TYW5 [48•]).

‡ KDM6B does not have reported TPR domains, though is roughly the same size as KDM6A and KDM6C.

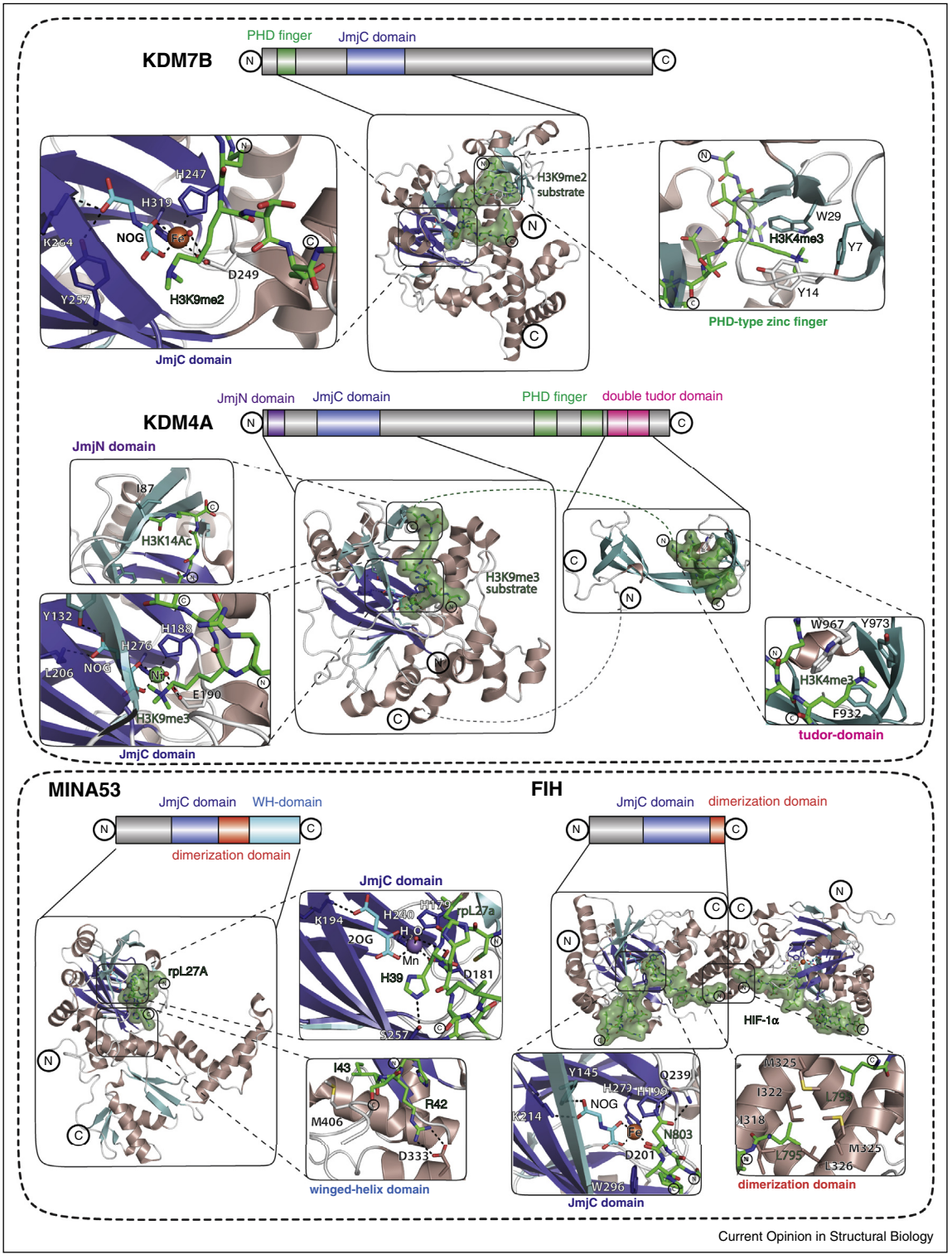
Abbreviations (alphabetical): *ARID*, AT-rich interaction domain; *eRF1*, eukaryotic release factor 1; *FBXL10/11*, F-box and leucine-rich repeat protein 10/11; *FIH*, factor inhibiting HIF-1α; *HIF-1α*, hypoxia inducible factor 1-alpha; *HIF1AN*, hypoxia inducible factor 1-alpha inhibitor; *HSPBAP1*, heat shock 27 kDa associated protein 1; *JARID*, Jumonji/AT-rich interaction domain-containing protein; *JHDM*, Jumonji-C domain-containing histone demethylase; *JmjC*, Jumonji-C; *JMJD*, Jumonji domain-containing; *JmjN*, Jumonji-N; *KDM*, lysine-specific demethylase; *LRR*, leucine rich repeat; *LUC7L2*, LUC7-like 2 pre-mRNA splicing factor; *MDC1*, mediator of DNA-damage checkpoint 1; *MINA(53)*, MYC-induced nuclear antigen (53); *NFATc1*, nuclear factor of activated T-cells calcineurin-dependent 1; *NO66*, nucleolar protein 66; *OHyW*, hydroxywybutosine; *p65*, nuclear factor NF-kappa-B p65 subunit; *PHD*, plant homeobox domain; *PHF2/8*, plant homeobox domain finger protein 2/8; *RBP2*, retinoblastoma binding protein 2; *RPL8/27*, 60S ribosomal protein L8/27; *TPR*, tetratricopeptide repeat; *TWY5*, tRNA wybutosine-synthesizing protein 5; *UTX/Y*, ubiquitously transcribed tetratricopeptide repeat, X/Y chromosome; *U2AF65*, U2 small nuclear ribonucleoprotein auxiliary factor 65-kDa subunit; *WH*, winged-helix; *yW-72*, 7-(α-amino-α-carboxypropyl)wyosine.

part via a positively charged patch formed by *C*-terminal α-helices [48•]. In the case of the ROXs, substrate binding is facilitated by the subfamily-characteristic *C*-terminal WH domain (Figure 2) [41•].

JmjC protein oligomerization

One difference observed between JmjC hydroxylases and KDMs is that the former oligomerize, usually as dimers, in a catalytically important manner (Supplementary Figure 3) [41•,49–51]. The FIH dimer interface is formed by four *C*-terminal α-helices (two from each monomer), which interlock primarily via hydrophobic interactions [49]. TYW5 also dimerizes via a *C*-terminal α-helix bundle [48•], though the anti-parallel orientation of its α-helical arrangement distinguishes it from FIH dimerization. The human and bacterial ROXs have conserved dimerization domains, but these are located between their JmjC and WH domains [40,41•,51].

Figure 2



Comparison of domain architecture and substrate binding between JmjC KDMs and hydroxylases. (*Upper panel*) Views from structures of KDM7B complexed with a H3K9me2K4me3 peptide fragment (PBD ID: 3KV4, [44•]), KDM4A complexed with a H3K9me3K14Ac peptide fragment (PBD ID: 2OQ6, [20•]), and KDM4A double tudor domains complexed with H3K4me3 peptide fragment (PDB ID: 2GFA, [45]). Zoomed views highlight key substrate binding interactions with active sites, the PHD non-catalytic domains of KDM7B, and the JmjN and double tudor non-catalytic domains of KDM4A. (*Lower panel*) Views from structures of MINA53 complexed with RPL27A peptide fragment (PBD ID: 4BXF, [41•]) and FIH complexed with a HIF-1α peptide fragment (PBD ID: 1H2K, [14•]). Zoomed views highlight key substrate interactions in the active site and the non-catalytic

JMJD6 oligomerization is more complex than for other studied hydroxylases. Structures for truncated JMJD6 present both dimeric and monomeric forms [52,53], though these do not fully reflect JMJD6 oligomerization. In solution, recombinant JMJD6 adopts different oligomeric states, including monomeric, trimeric, pentameric and larger oligomeric forms [54–57]. JMJD6 oligomerization, which is affected by the presence/absence of its polyserine domain, is proposed to play a role in both catalysis and subcellular localization of JMJD6 [55]. JMJD6, like TYW5, has a positively charged surface patch, which may enable RNA binding [53].

Despite its assignment as a KDM [32], JMJD5 more closely resembles the JmjC hydroxylases (see below). Structures of *N*-terminally truncated JMJD5 constructs reveal that the DSBH is located immediately at the *C*-terminus [50,58]; yet, *N*-terminally truncated JMJD5 exists in both monomeric and dimeric oligomerization states in solution [58], suggesting JMJD5 oligomerizes via a different mode than FIH and TYW5 [14•,48•,49].

In contrast to the hydroxylases, KDM oligomerization is not well characterized, likely in part due to their size and typical multi-domain architecture. Currently, there are only crystal structures for truncated JmjC KDM constructs, none of which reveal oligomerization. Yet, solution studies indicate full-length KDM3A forms a homodimer via interactions between a Zn finger motif and its JmjC domain [59]. In the case of the KDM4 subfamily, cell-based immunoprecipitation experiments suggest that full-length KDM4A, C, and D are capable of forming homo-oligomers, while KDM4A/C can hetero-oligomerize [60].

JmjC active site substrate binding

Differences in substrate binding within their active sites distinguish JmjC KDMs from hydroxylases. To accommodate N^{ε}-methylated lysine residues, the KDMs tend to have a deeper and sometimes narrower substrate binding pocket than the hydroxylases (Figure 3a). The KDMs typically have a hydrophobic region in their active site (e.g. as formed by V231, Y234, L236, F250, G331, and N333 in KDM7B), which interacts with the hydrophobic N^{ε}-methyl lysine side chain and N^{ε}-methylated group (Figure 3b) [44•]. While such hydrophobic interactions are generally characteristic of KDMs, there are variations in the modes of substrate binding/recognition between KDM subfamilies. For example, KDM2A and KDM7B appear to adopt a binding mode that positions the target N^{ε}-methyl lysine residue within a relatively deep and narrow binding pocket [44•,61]. In contrast, KMD4A and KDM6A adopt a binding mode where the substrate binds more on the surface of the protein, and the target N^{ε}-methyl lysine residue binds in a relatively open pocket [20•,37]. Although other factors are likely involved, it is possible that the apparently more spacious binding pockets of the KDM4/KDM6 subfamilies enables them to accommodate the more bulky trimethylation state [20•,37], which the KMD2/KMD7 subfamilies do not accommodate [61,62] (though KDM2A has been crystallized with a non-substrate trimethyllysine residue in its active site [61]).

JmjC hydroxylases in general appear to have more open binding sites than the KDMs (Figure 3a). Further, they bind their target residues primarily through main and side chain hydrogen bonds, rather than the principally hydrophobic interactions used by KDMs (Figure 3b). Such binding is proposed to enable stereoselective hydroxylation [14•]. Stereoselectivity is likely not so important with KDMs because methyl group oxidation leads to achiral formaldehyde. KDMs also (normally) catalyze sequential demethylations [63]; the hydrophobic interactions involved in KDM substrate residue binding may enable flexibility, which is undesirable in stereoselective hydroxylase catalysis. Interestingly, the coordination position from which the Fe(IV)=O species reacts appears to vary with the JmjC hydroxylases (e.g. it is different for the FIH-catalyzed Asn hydroxylation compared to ROX-catalyzed His hydroxylation) [41•], a difference proposed to reflect a branchpoint in JmjC oxygenase evolution, with the ROXs being closer to the KDMs than other hydroxylases (e.g. FIH) [41•].

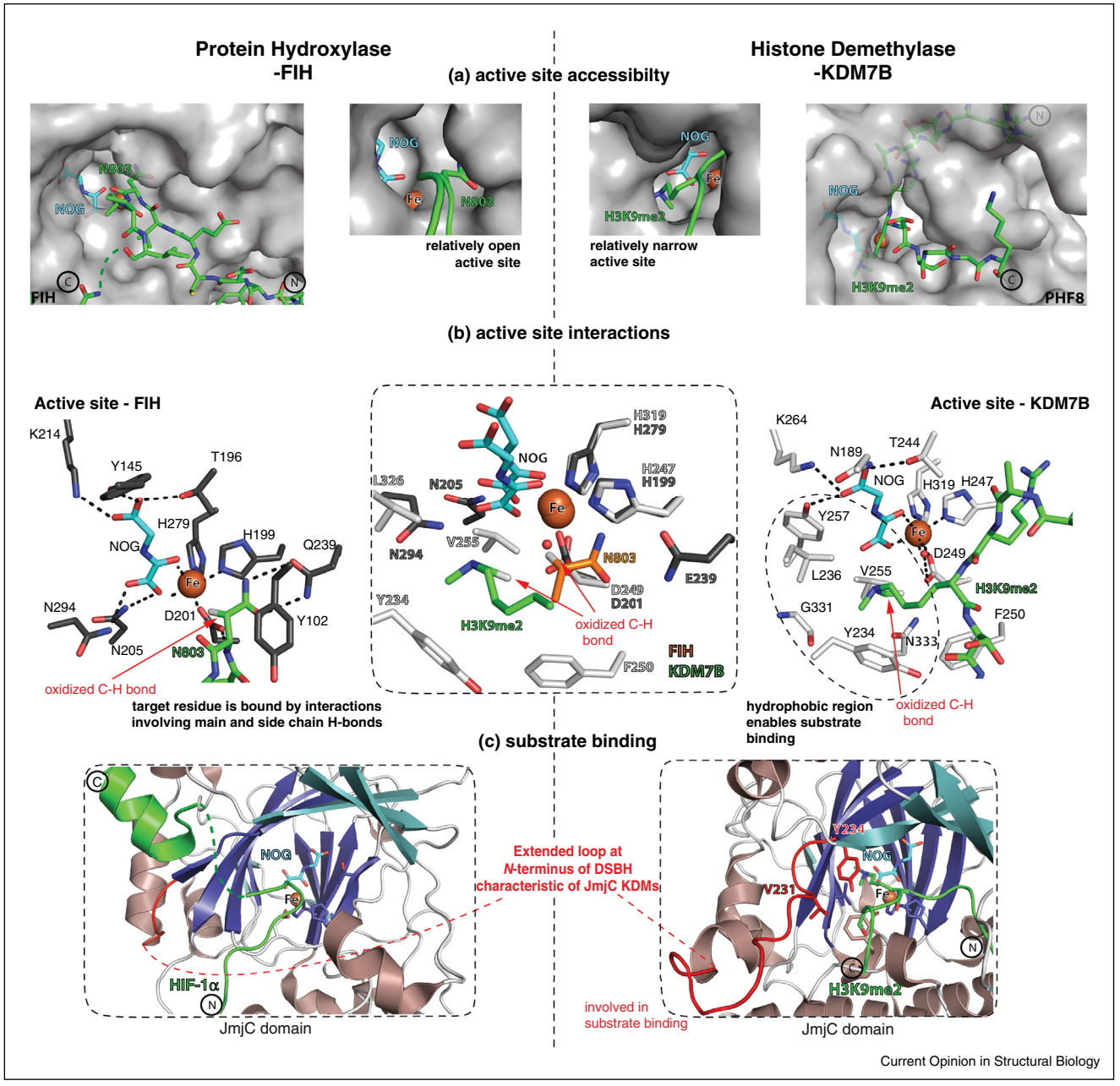
Another typical difference in active site substrate binding between JmjC hydroxylases and KDMs is the presence of an extended flexible loop region, immediately *N*-terminal to βI of the DSBH, that is involved in binding target N^{ε}-methylated lysine residues (Figure 3c). For KDM7B, this loop (residues 221–234) contains V231 and Y234, which form stabilizing hydrophobic interactions with the target residue as described above. This loop is observed to be involved in substrate binding in substrate-bound crystal structures of KDM2A, KDM4A, and KDM6A [20•,37,61]. However, hydroxylases tend to lack this extended loop, as exemplified by FIH (Figure 3c) [14•]. The available evidence is that hydroxylases bind their substrates with an (approximately) conserved *N*-to-*C* directionality with respect to their active site, whereas substrate binding directionality appears more variable for the KDMs [41•].

Controversial assignments of JmjC oxygenases

Some JmjC oxygenases are assigned as both KDMs and hydroxylases. These include the histidinyl hydroxylases

(Figure 2 Legend Continued) winged-helix (WH) domain of MINA53 and the dimerization domain of FIH. Substrate peptide fragments are shown as *green* sticks. Note in some cases, Mn(II)/Ni(II) substitutes for Fe(II) and N-oxalylglycine (NOG) substitutes for 2OG in order to enable crystallographically stable complexes. Abbreviations are defined in Table 1.

Figure 3



Structural comparisons of active site accessibility/interactions and substrate recognition/binding of JmjC oxygenases. Comparison of structures of FIH complexed with the *C*-terminal transactivation domain HIF-1α peptide fragment (PBD ID: 1H2K, [14•]) and KDM7B complexed with an H3K9me2 peptide fragment (PBD ID: 3KV4, [44•]). **(a)** Surface views of FIH and KDM7B reveal differences in the active site topologies/accessibilities of hydroxylases compared to KDMs. The available evidence is hydroxylases (*left*) have a relatively shallow, wide/open active site whereas KDMs (*right*) generally have a relatively narrow, deep active site. **(b)** Comparison of substrate interactions as shown by stick representations of key residues of FIH (*black*) and KDM7B (*white*). KDMs (*right*) tend to have a hydrophobic active site region, formed by V231, Y234, L236, F250, G331, and N333 in KDM7B, important in binding the N^{ε}-methylated lysine. Hydroxylases (*left*) bind their substrates by both main and side chain hydrogen bonds. FIH and KDM7B active sites are overlaid in a central box. **(c)** The role of an extended flexible loop (*red*), situated *N*-terminal to β1 of the DSBH, in substrate binding. This extended flexible loop tends to play an important role in KDM active site N^{ε}-methylated lysine binding, as exemplified by the loop in KDM7B (*right*). In contrast, the hydroxylases tend to lack (or have a much shorter) extended flexible loop as shown by FIH (*left*). Note that JMJD6 is an exception (see main text). Substrate peptide fragments are shown as *green* sticks. Abbreviations are defined in Table 1.

MINA53 and NO66, which were first assigned as KDMs [30,31], though later shown to catalyze (*3S*)-histidinyl hydroxylation of ribosomal proteins RPL27a and RPL8, respectively [24•]. While it is possible MINA53 and NO66 have dual functions, independent attempts to confirm their KDM activity using MS-based assays have not yielded results supporting this assignment [24•,64]. Further, structural characterizations support the assignment of the human ROXs as hydroxylases, for reasons including (i) the presence of a dimerization domain, (ii) that their active sites are relatively open, (iii) that their substrates are bound by polar/hydrogen bonding interactions that enable stereoselective hydroxylation, (iv) clear homology with the prokaryotic protein hydroxylase ycfD and (v) the lack of an extended flexible KDM substrate binding loop *N*-terminal to DSBH β1 typically observed in KDMs (Supplementary Figure 1) [24•,41•,51]. JMJD6 likely has the most conflicting reports of any JmjC oxygenase. JMJD6 was initially (likely) mis-assigned as the phosphatidylserine receptor [65]. It was subsequently characterized as a histone N-methyl arginyl demethylase acting on H3R2me1 and H4R3me1 [26•], then as a lysyl hydroxylase acting on splicing-associated proteins (e.g. U2AF65 and LUC7L2) and histones [22•,27,28]. JMJD6 is also reported to act on other proteins, including via lysyl hydroxylation of p53 [66]. Evidence for 'precise' hydroxylase-type catalysis by JMJD6 comes from studies on the stereochemistry of its catalysis. This work reveals production of (5*S*)-hydroxylysine [27], contrasting with the other reported lysyl C-5 hydroxylase, procollagen lysyl hydroxylase, which catalyses production of the (5*R*)-hydroxylated product [67]. As yet, there are no reports of JMJD6 substrate complex structures, but the available evidence implies JMJD6 is more similar to the JmjC hydroxylases than KDMs based on (i) closer sequence similarity to the JmjC hydroxylases, (ii) its ability to oligomerize, (iii) its relatively open active site, and (iv) topological similarity with some JmjC hydroxylases (Supplementary Figure 1) [14•,41•,48•]. Interestingly, JMJD6 contains an extended flexible loop immediately *N*-terminal to DSBH βI [52,53], which is typically observed in KDMs, and so the possibility that it also functions as a demethylase cannot be ruled out. A detailed review of JMJD6 is given by Böttger *et al.* [68]. Although JMJD5 is a reported KDM [32], several studies have been unable to demonstrate JMJD5-catalyzed demethylation [33,50,58]. Structural analyses of JMJD5, as given here and by others, imply JMJD5 likely functions as a hydroxylase due to structural similarities with TYW5 and FIH (Supplementary Figure 1) [50,58]. However, no direct hydroxylation activity has yet been reported, though NFATc1 (nuclear factor of activated T-cells calcineurin-dependent 1) is proposed as a JMJD5 substrate [33].

Some JmjC oxygenases are proposed to be 'pseudo' enzymes, that is they are closely related to demonstrated enzymes, but are catalytically inactive. In some predicted JmjC proteins, the HxD/E···H Fe(II)-binding triad is incompletely conserved, for example in JARID2 (predicted to have only one conserved His residue), KDM7C (HxD···Y), and Hairless (CxE···H) [6,62,69]. It is possible that these enzymes bind Fe(II) with only two residues, as supported by FIH mutagenesis studies [70], or that residues in unanticipated regions are involved in metal chelation. Interestingly, biochemical and crystallographic studies reveal that these proposed 'pseudo' enzymes may still bind histones. For example, KDM7C binds H3K4me3/2 in a manner related to its PHD domain [62,71], and Hairless is proposed to have KDM activity acting on H3K9me2/1 [69]. Further, there are reports of KDM7C catalytic activity on H3K9me2 via protein kinase A-dependent phosphorylation and demethylation of ARID5B [72,73].

Future perspectives

Since the identification of the JmjC domain in JARID2 (Jumonji), there have been significant advances in the assignment of biochemical and biological functions for the JmjC oxygenases. Their now established roles in chromatin biology and other aspects of epigenetic regulation means that several JmjC KDMs are of interest from a therapeutic perspective, particularly for cancer treatment. Many human JmjC proteins are assigned catalytic roles as protein oxygenases, with a notable exception being TYW5, which acts on tRNA. While most of the human JmjC KDMs act on histones, there is the possibility that they can act on non-histone proteins, as apparently do most JmjC hydroxylases. Crystallographic analyses have revealed structural differences between the KDMs and hydroxylases, which are of value in predicting functional assignments of uncharacterized JmjC oxygenases. To date, relatively few structures of JmjC oxygenases with substrates have been reported, and none with intact protein substrates. Given the size and complexity of JmjC oxygenases and their associated substrates, future efforts can focus on obtaining such structures, which are of particular interest to research aiming to understand the roles of the JmjC oxygenases in the modification of nucleosomes and translational machinery. Biochemical and structural characterization of atypical members of the JmjC subfamily, including potential 'pseudo' enzymes, like JARID2, may be of interest, especially since the history of 2OG oxygenases suggests that unexpected reactions and biological roles may emerge.

Conflict of interest statement

Nothing declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sbi.2016.05.013.

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